Figure 1

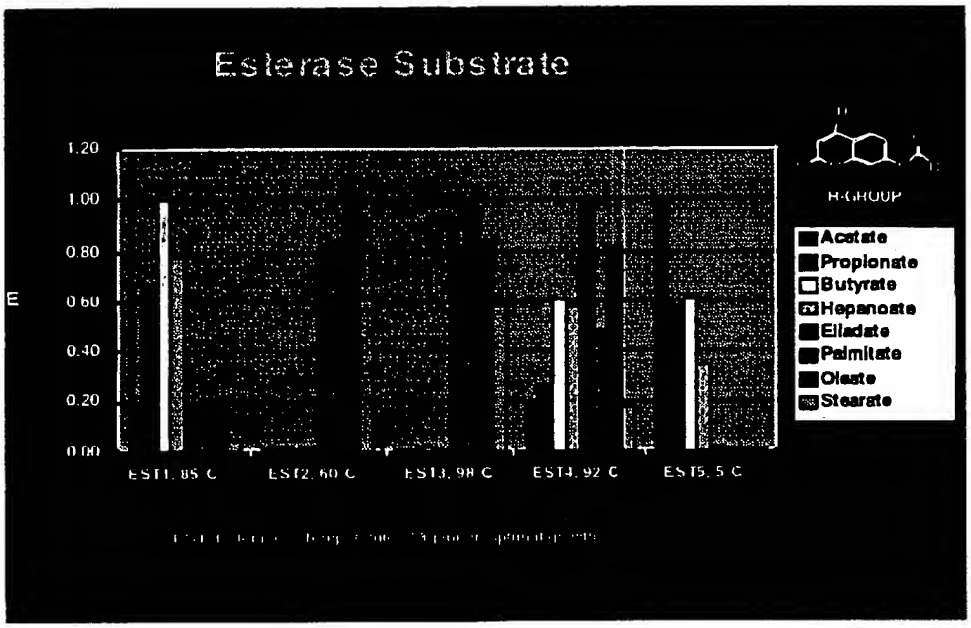
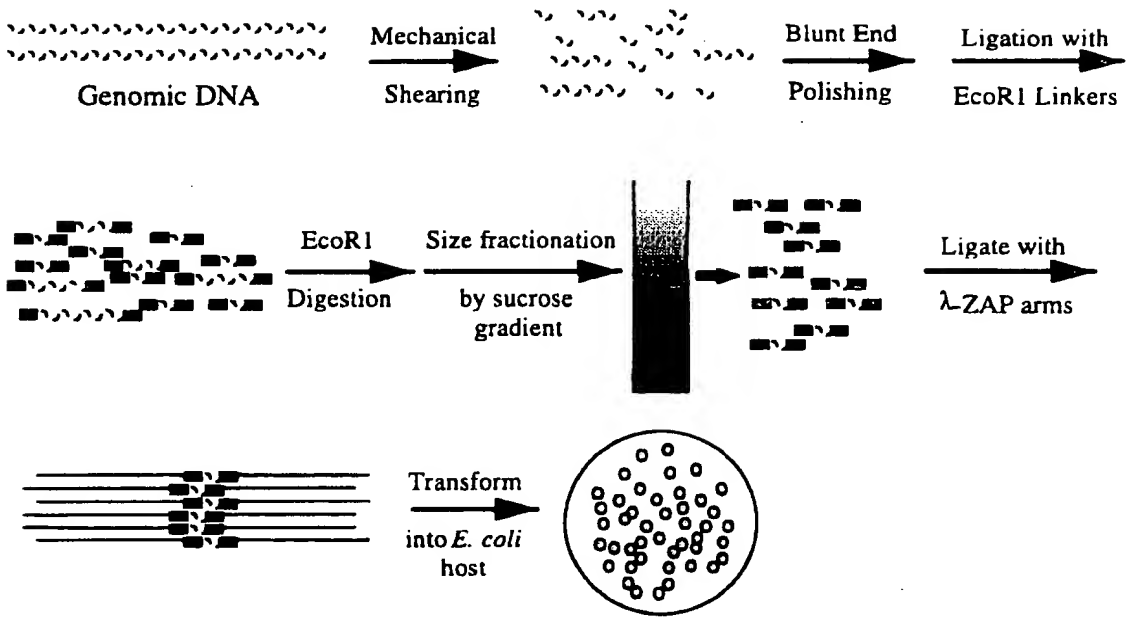

Esterase Substrate
1.20
1.00
0.80
0.60
0.40
0.20
0.00
EST1, 85 C
EST2, 60 C
EST3, 98 C
EST4, 92 C
EST5, 5 C
R-GROUP
Acetate
Propionate
Butyrate
Hepanoate
Eiladate
Palmitate
Oleate
Stearate

Figure 2.


Genomic DNA
Mechanical
Shearing
Blunt End
Polishing
Ligation with
EcoR1 Linkers
EcoR1
Digestion
Size fractionation
by sucrose
gradient
Ligate with
λ-ZAP arms
Transform
into E. coli
host

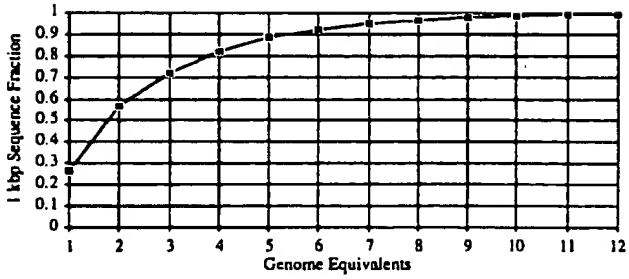

1 kbp Sequence Fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1 2 3 4 5 6 7 8 9 10 11 12
Genome Equivalents

Figure 3.

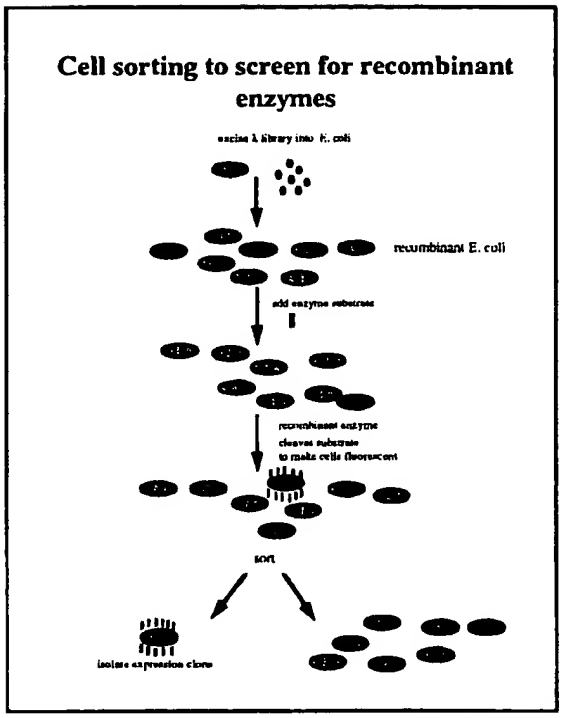
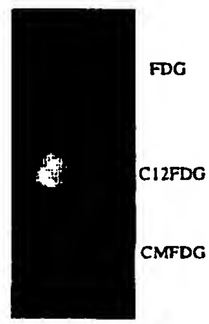

Cell sorting to screen for recombinant enzymes
recombinant E. coli
add enzyme substrate
recombinant enzyme
cleaves substrate
to make cells fluorescent
sort
isolate expression clone

Figure 4.

β-Gal clone with different substrates

- cells were stained with FDG, CMFDG or C12FDG, incubated for 30 min. at 70°C, spotted onto a slide and exposed to UV light.
- bright spot indicates staining of cells



E. coli expressing β -Gal from Sulfulobus spec. was grown over night. Cells were centrifuged and substrate was loaded with deionised water. After 5 min. cells were centrifuged and transferred into HEPES buffer and heated to 70°C for 30 min.. Cells were spotted onto a slide and exposed to UV light.

Figure 5

Figure 6

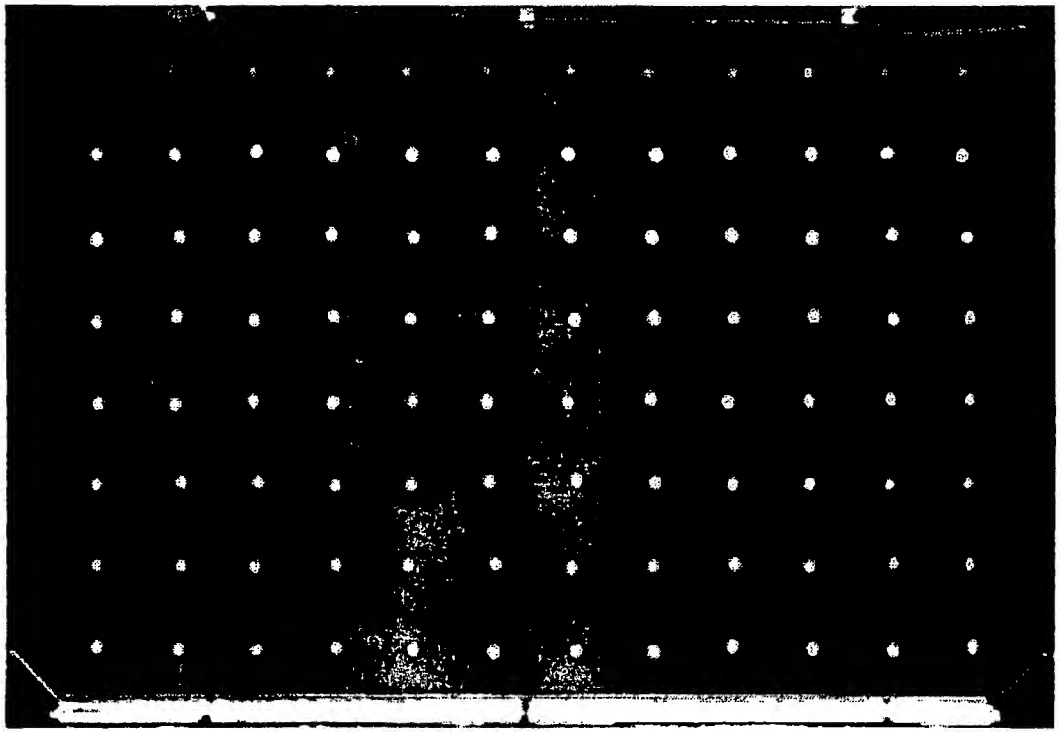
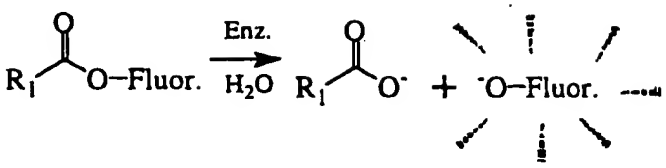


Figure 7

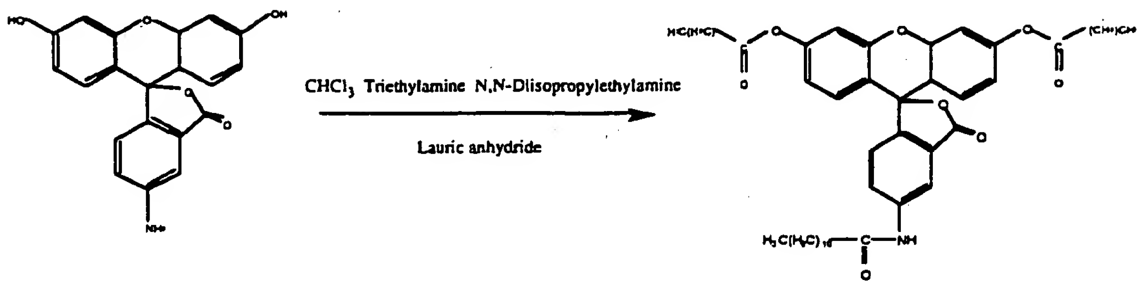


Principle type of fluorescence enzyme assay of deacylation.

Figure 8

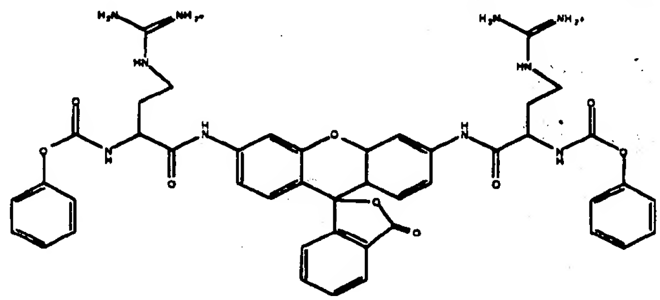
Staining of β-galactosidase clones from the hyperthermophilic archaeon *Sulfolobus solfataricus* expressed in *E.coli* using C_{12}-FDG as enzyme substrate.

Figure 9



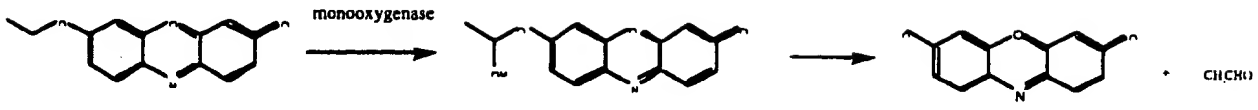
Synthesis of 5-dodecanoyl-aminofluorescein-di-dodecanoic acid

Figure 10



Rhodamine protease substrate.

Figure 11



Compound and process that can be used in the detection of monoxygenases

NA Library

Combinatorial Enzyme Development

(Natural + Non-natural Evolution)

Search Nature + Improve Nature = Desired Enzyme

Enzyme Library

Enzyme 1
Enzyme 2
Enzyme 3
Enzyme 4
Enzyme 5
Enzyme 6
Enzyme 7
Enzyme 8
Enzyme 9
Enzyme 10
Enzyme n

Select Enzyme

Directed Evolution

Enz. 2 → Enz. 2'
Enz. 7 → Enz. 7' → New Enzyme
Enz. 33 → Enz. 33'

ID via High Throughput Screening

ID via Enzyme Characterization

ID via Mutation / Selection

URE 12

Bypassing Barriers to Directed Protein Evolution

(Barrier = Capacity limit of directed evolution system)

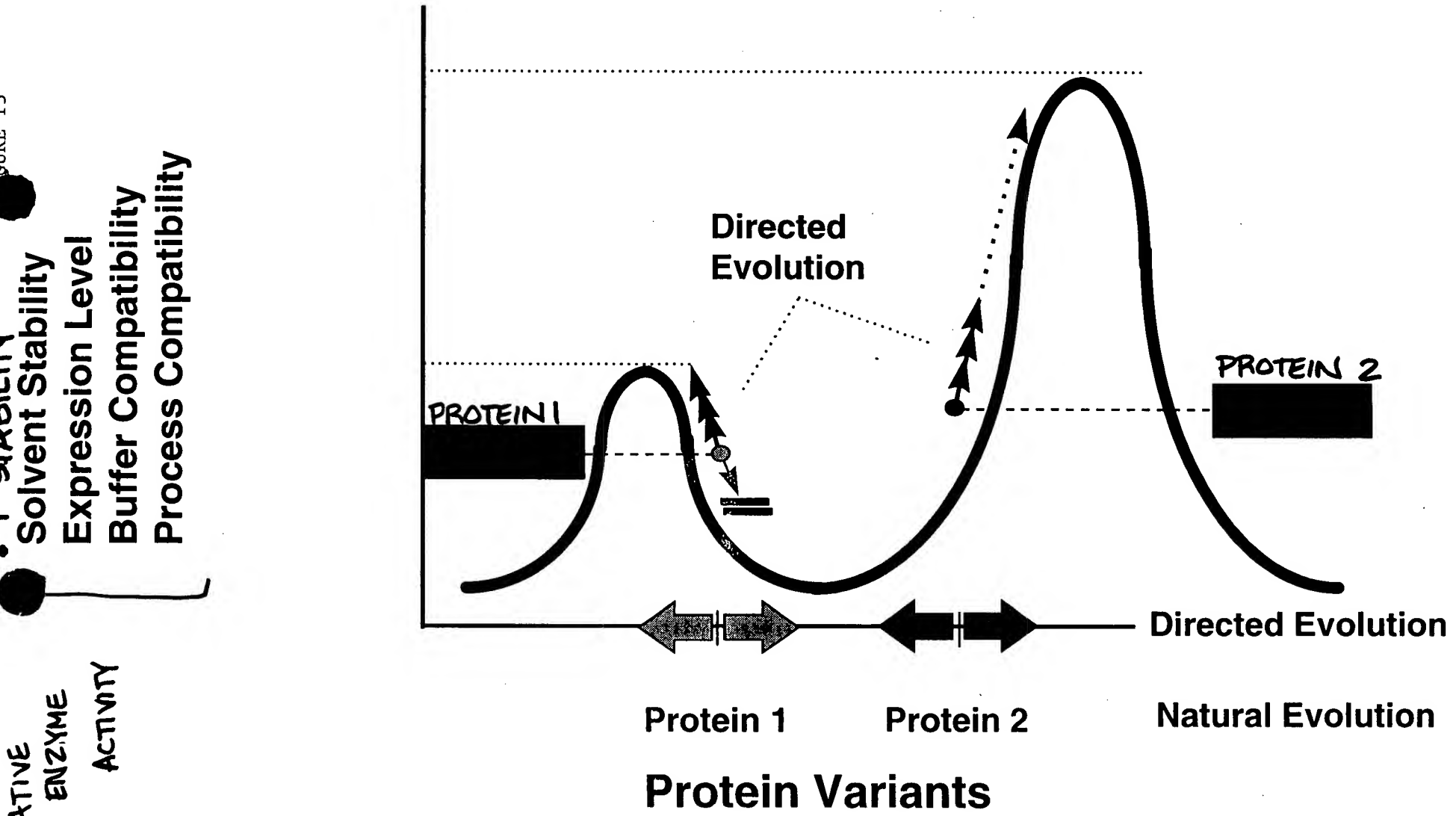


FIGURE 13